9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator materials used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity data--the number of untreated controls with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness data--the number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). Differences among treatments may be demonstrated by use of additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on wood blocks as a result of treatment.

9.1.7 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

1/ Cultures of A. niger (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

2/ Cultures of P. variabile (NRRL-3765, ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, Northern Utilization Research and Development Division, 1815 North University Street, Peoria, Ill.; or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

3/ Triton X-100, Rohm & Haas Co. Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate (Aerosol OT solid A-349, Fisher Scientific Co.).

4/ For detailed instructions: Tuite, John, Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

Item 5 - Glass Slide Mildew Fungicidal Test Method

1. Scope.

1.1 Products intended for use to kill fungi which cause mold and mildew growth should be tested to demonstrate "cidal" effectiveness. This method is designed to determine the effectiveness of products intended to kill mildew organisms on hard, nonporous surfaces. Residual effectiveness if intended or claimed must be demonstrated using other tests (i.e., Fabric Mildew Fungistatic Test Method, Item 1; Hard Surface Mildew Fungistatic Test Method, Item 2; Leather Mildew Fungistatic Test Method, Item 3; Wood Block Mildew Fungistatic Test Method, Item 4) depending on the nature of the surfaces or articles on which the product is intended to be used. This method is especially applicable for testing products applied as sprays or formulated as pressurized sprays.

2. Summary of Method.

2.1 This method is conducted using specially prepared sterile square glass slides which are seeded with a standardized spore suspension of the test organism. The slides are sprayed individually with the test fungicide and then placed into large test tubes containing culture medium plus a fungicide neutralizer for incubation and subsequent evaluation for the presence or absence of fungal growth.

3. Apparatus.

3.1 Glassware: Lipless 32 x 200 mm test tubes with plugs. Petri dishes 15 x 100 mm. Flasks with plugs. All glassware sterilized two hours in hot air oven at 180°C.

3.2 Transfer loop (or equivalent device) which will deliver approximately 0.01 ml of spore suspension.

3.3 Racks and baskets: Suitable for holding test tubes.

3.4 Microscope slides: Noncorrosive (25 x 25 mm) slides placed individually in a petri dish matted with two pieces of 90 mm diameter filter paper (Whatman No. 1 or equivalent) and placed in a hot air oven for sterilization.

3.5 Tissue grinder (Homogenizer) No. 4288B Arthur H. Thomas Co.

3.6 Counting chamber suitable for determining spore concentration.

4. Reagents and Materials.

4.1 Distilled water or water of equal purity.

4.2 Neopeptone--neutralizer broth: prepare by dissolving 0.7g lecithin and 5.0g sorbitan monooleate 1/ in 400 ml of hot water and boiling until dissolved. Add 1.0g sodium thiosulfate, 10g neopeptone and 20g dextrose, and sufficient water to make one liter of culture medium. The pH of the medium will be approximately 7.2 after autoclaving.

4.4 Saline solution as 0.85% sodium chloride and 0.05% isooctylphenoxypolyethoxyethanol 2/ in distilled water.

5. Test Fungus.

5.1 Aspergillus niger (ATCC 6275) 3/. Maintain stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar and 1 liter distilled water). Incubate stock culture for 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungicide: A sufficient number of dosages of the test fungicide should be evaluated in order to determine the minimum effective dosage.

6.2 Untreated Control: Two untreated slides are included for determining the validity of the test.

6.3 Standard Fungicide: A fungicide registered for use on similar surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungicide.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of Conidial Suspension: Conidial suspensions of the fungus are prepared by washing the spores from the surface of 7- to 10-day old neopeptone agar cultures with sterile saline solution. The spore suspension is poured into a heat sterilized tissue grinder and the piston reciprocated several times to break up the spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml by adding sterile diluent. Determine spore concentration with a counting chamber 4.

7.2 Inoculation: Agitate spore suspension to disperse spores evenly throughout, transfer approximately 0.01 ml of the spore suspension by means of a transfer loop onto each 25 mm square sterile test slide (contained in a petri dish) and spread evenly over the upper surface. Cover the dish immediately and repeat the procedure until twelve slides have been prepared (use two slides as controls). Allow all slides to dry for 40 minutes at 37°C or let stand several hours at room temperature.

7.3 Treatment: Spray ten inoculated slides with the test product concentration at a specified distance to obtain the desired degree of wetness. Immediately after treatment, drain excess liquid from slides and maintain in a petri dish for an exposure of one minute. NOTE: Products which are capable of keeping surfaces totally wet for longer than one minute under actual use conditions, should be tested under a longer exposure time. To determine the duration of such an increased exposure time the following test procedure should be employed. Tests must utilize a hard nonporous surface (e.g., glass, metal, or porcelain) of at least one square foot in area which are treated in accordance with the proposed label directions for use. The test surface(s) must be positioned vertically, unless the product is intended solely for use on horizontal surfaces (e.g., floors) in which case horizontal positions must be used. The test must be conducted at a temperature

of 20 to 25°C and a relative humidity of 50% or less. The length of time (in seconds) from application to when any portion of the treated surface, begins to appear dry should be recorded. The average length of this drying time for a minimum of three replicates shall serve as the basis for determining the increased treatment exposure time. Products which keep surfaces totally wet for longer than 10 minutes should utilize a 10 minute exposure time.

7.4 Incubation: Transfer each slide by means of flamed forceps to separate 32 x 200 mm test tubes containing 20 ml of neopeptone neutralizer broth. Shake culture medium thoroughly. Transfer two unsprayed slides, as viability controls, to individual culture tubes in the same manner. Incubate all tubes at 25°C for at least three days.

8. Determination of Results.

8.1 Evaluation: The presence or absence of fungal growth, after 3 days, is the criterion for determining "cidal" effectiveness of the test product. For a valid test, fungal growth must be present in both viability control replicates.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated with the intended label claims. Products which pass this test may be labeled as fungicides or mildewcides which kill mold and mildew organisms. If the product is not tested for residual effectiveness, the labeling must state "non-residual" or "kills on contact."

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungicide used).

9.1.2 Dosage rates (specify whether in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Effectiveness data--the number of replicates with fungal growth for each treatment being evaluated (including untreated controls).

9.1.5 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change. For example: the duration of any exposure time which is longer than 1 minute exposure specified in section 7.3, plus the results of the drying time test used to support the change in exposure time should be reported.

Footnotes

1/ Tween-80, ICI United States, Agricultural Division, Wilmington, Del. 19898.

2/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable agent such as dioctyl sodium sulfosuccinate (Aerosol OT solid A-349, Fisher Scientific Co.).

3/ Cultures of A. niger (ATCC 6275) may be obtained from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

4/ For detailed instructions: Tuite, John. 1969. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing Co., Minneapolis, Minn. Pp. 183-184.

Item 6 - Use-Dilution Mildew Fungicidal Test Method

1. Scope.

1.1 Products intended for use to kill fungi which cause mold and mildew growth should be tested to demonstrate "cidal" effectiveness. This method is designed to determine the effectiveness of products intended to kill mildew organisms on hard non-porous surfaces. Residual effectiveness, if intended, must be demonstrated using other tests (i.e., Fabric Mildew Fungistatic Test Method, Item 1; Hard Surface Mildew Fungistatic Test Method, Item 2; Leather Mildew Fungistatic Test Method, Item 3; Wood Block Mildew Fungistatic Test Method, Item 4) depending on the nature of the surfaces or articles on which the product is intended to be used. This method is especially applicable for testing products which are applied by non-spray methods of application (wiping, mopping, etc.).

2. Summary of Method.

2.1 This method is conducted using polished cylinders (penicillin cups) as carriers which are seeded with a standardized spore suspension of the test organism. After carriers are dried, they are immersed in the use-dilution of the product, and then placed in test tubes containing culture medium plus fungicide neutralizer. After incubation, evaluation as the presence or absence of fungal growth is made.

3. Apparatus.

3.1 Glassware: Lipless 25 by 150 mm test tubes with cotton plugs. Petri plates 15 by 100 mm with filter paper. Erlenmeyer flasks (250 and 1000 ml) with cotton plugs.